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60/457,053 24 March 2003 (24.03.2003) US
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(54) Title: ENHANCED ACCUMULATION OF CAROTENOIDS IN PLANTS

(57) Abstract: The present invention relates to polynucleotides and their use in methods of increasing the carotenoid content of seeds. In particular the invention provides a polynucleotide comprising: (a) a region which comprises as operably linked components (i) a promoter which provides for seed preferred expression; and (ii) a nucleotide sequence derived from a bacterium which sequence encodes a carotene desaturase; and (iii) a transcription termination region; and (b) a further region which comprises as operably linked components (i) a promoter which provides for seed preferred expression; and (ii) a nucleotide sequence encoding a phytoene synthase which sequence is derived from maize (*Zea* sp.) or rice (*Orzya* sp.); and (iii) a transcription termination region. The disclosed polynucleotides are particularly suitable for use in production of rice seed which comprise high amounts of coloured carotenoids.

WO 2004/085656 A3

INTERNATIONAL SEARCH REPORT

International Application No
/GB2004/001241

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C12N15/53 C12N15/54 C12N15/62 A01H5/10
A01H5/00 C07C403/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/53768 A (POTRYKUS INGO ; BEYER PETER (DE); GREENOVATION PFLANZENBIOTECHNO (DE)) 14 September 2000 (2000-09-14) page 7, line 20 - page 9, line 9; figure 4	1-37
X	WO 98/06862 A (SHEWMAKER CHRISTINE K ; CALGENE INC (US)) 19 February 1998 (1998-02-19) page 10, line 23 - page 11, line 1	1-37
X	WO 02/16583 A (MAXYGEN INC ; LIU LU (US); ZHU GENHAI (US)) 28 February 2002 (2002-02-28) page 57, line 19 - page 59, line 22; claim 7	1-37
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
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- *&* document member of the same patent family

Date of the actual completion of the international search

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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROEMER S ET AL: "ELEVATION OF THE PROVITAMIN A CONTENT OF TRANSGENIC TOMATO PLANTS" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 18, no. 6, June 2000 (2000-06), pages 666-669, XP001155851 ISSN: 1087-0156 abstract	
A	BEYER P ET AL: "GOLDEN RICE: INTRODUCING THE BETA-CAROTENE BIOSYNTHESIS PATHWAY INTO RICE ENDOSPERM BY GENETIC ENGINEERING TO DEFEAT VITAMIN A DEFICIENCY" JOURNAL OF NUTRITION, WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, PHILADELPHIA, PA,, US, vol. 132, no. 3, March 2002 (2002-03), pages 506S-510S, XP001069128 ISSN: 0022-3166 the whole document	
A	KUMAGAI M H ET AL: "CYTOPLASMIC INHIBITION OF CAROTENOID BIOSYNTHESIS WITH VIRUS-DERIVED RNA" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 92, 1 February 1995 (1995-02-01), pages 1679-1683, XP002012921 ISSN: 0027-8424 abstract	
A	BARTLEY G E ET AL: "TWO ARABIDOPSIS THALIANA CAROTENE DESATURASES, PHYTOENE DESATURASE AND DZETA-CAROTENE DESATURASE, EXPRESSED IN ESCHERICHIA COLI, CATALYZE A POLY-CIS PATHWAY TO YIELD PRO-LYCOPENE" EUROPEAN JOURNAL OF BIOCHEMISTRY, BERLIN, DE, vol. E-259, no. E-1/2, 1999, pages 396-403, XP000925505 ISSN: 0014-2956 abstract	
P,X	RAVANELLO MONICA P ET AL: "Coordinate expression of multiple bacterial carotenoid genes in canola leading to altered carotenoid production." METABOLIC ENGINEERING. OCT 2003, vol. 5, no. 4, October 2003 (2003-10), pages 255-263, XP002295220 ISSN: 1096-7176 abstract	1-37

INTERNATIONAL SEARCH REPORT

international application No.

PCT/GB2004/001241

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☒ contained in the international application as filed
 - ☐ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purpose of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0053768	A	14-09-2000	DE 19909637 A1	07-09-2000
			AU 3285500 A	28-09-2000
			BR 0009258 A	20-11-2001
			CA 2362448 A1	14-09-2000
			CN 1347454 T	01-05-2002
			WO 0053768 A1	14-09-2000
			EP 1159428 A1	05-12-2001
			HU 0105167 A2	29-04-2002
			ID 29890 A	18-10-2001
			JP 2002537841 A	12-11-2002
			PL 350535 A1	16-12-2002
			ZA 200106948 A	22-11-2002
WO 9806862	A	19-02-1998	AU 4058497 A	06-03-1998
			BR 9713462 A	28-03-2000
			CA 2261577 A1	19-02-1998
			CN 1227609 A	01-09-1999
			EP 0925366 A1	30-06-1999
			JP 2001505409 T	24-04-2001
			US 2002092039 A1	11-07-2002
			WO 9806862 A1	19-02-1998
			US 6429356 B1	06-08-2002
WO 0216583	A	28-02-2002	AU 8499701 A	04-03-2002
			CA 2421059 A1	28-02-2002
			CN 1468313 T	14-01-2004
			EP 1317535 A2	11-06-2003
			WO 0216583 A2	28-02-2002
			US 2002132308 A1	19-09-2002